Reference: *Biol. Bull.* **182**: 341–347. (June, 1992)

Quantitative Analysis by Reverse Phase High Performance Liquid Chromatography of 5-Hydroxytryptamine in the Central Nervous System of the Red Swamp Crayfish, *Procambarus clarkii*

GUNDERAO K. KULKARNI AND MILTON FINGERMAN

Department of Ecology, Evolution, and Organismal Biology, Tulane University, New Orleans, Louisiana 70118

Abstract. The concentrations of 5-hydroxytryptamine (5-HT) in central nervous organs of the red swamp crayfish, *Procambarus clarkii,* were determined by reverse phase high performance liquid chromatography (RP-HPLC) with electrochemical detection. The quantity ranged between 54 and 168 pg/mg wet weight of tissue. The amount is highest in the brain, followed in decreasing order by the thoracic ganglia, subesophageal ganglion, eyestalks, and abdominal nerve cord. Significant increases in the levels of 5-HT in the eyestalks, brain, subesophageal ganglion, and thoracic ganglia occurred in crayfish exposed for three days to continuous light on a white background, whereas the 5-HT levels in these tissues decreased in crayfish kept in darkness. Electrical stimulation of central nervous organs *in vitro* produced significant decreases in the levels of 5-HT. Fenfluramine (5-HT releaser), 5,6-DHT (5-HT neurotoxin), and reserpine (5-HT depletor) induced significant decreases in the 5-HT levels in the portions of the central nervous system tested.

Introduction

The biogenic amines, norepinephrine, dopamine, histamine, octopamine, 5-hydroxytryptamine (5-HT), and gamma aminobutyric acid, function as neurotransmitters in various animals (Werman, 1966; Gerschenfeld, 1973; Krnjevic, 1974; Fingerman, 1985), and have been found in crustacean central nervous organs (Beltz and Kravitz, 1983; Elofsson, 1983; Laxmyr, 1984; Fingerman, 1985; Sandeman *et al.,* 1988). Aréchiga *et al.* (1990) showed that the species used in this study, the red swamp crayfish *Procambarus clarkii,* contains, in the lamina ganglionaris of its eyestalks, a set of axons with 5-HT-like immunoreactivity. These investigators also found that the responsiveness of the retinal photoreceptors of this crayfish to light is enhanced by exposure to 5-HT, both *in vivo* and *in vitro.* With respect to other crayfishes, 5-HT-containing neurons have been reported in the optic lobes and proto-, deuto-, and tritocerebral regions of the brain of *Pacifastacus leniusculus* (Myhrberg *et al.,* 1979; Elofsson, 1983), *Orconectes virilis* (Sandeman and Sandeman, 1987), and *Cherax destructor* (Sandeman *et al.,* 1988).

Kulkarni *et al.* (1991) have shown that 5-HT stimulates oocyte maturation in *Procambarus clarkii.* This observation and the earlier study of Aréchiga *et al.* (1990) have led us to determine, for the first time, the quantity of 5-HT in the central nervous system of this crayfish. In addition, we have determined the effects, on the 5-HT concentration in components of the central nervous system of (1) continuous exposure to light or darkness, (2) *in vitro* electrical stimulation, and (3) pharmacological agents known to affect 5-HT levels in vertebrates.

Received 21 October 1991; accepted 26 March 1992.

Materials and Methods

Animals

Red swamp crayfish, *Procambarus clarkii,* were purchased from a local seafood dealer and maintained in the laboratory at 24°C in a recirculating freshwater system. They were acclimatized to the laboratory conditions (12:12 L:D) for at least two days before being used in an experiment. Medium sized (carapace length 40–50 mm), intermolt (Stage C_4, Reddy *et al.,* 1990) crayfish of both

sexes were used. The crayfish were fed commercial crayfish food. Fiddler crabs, *Uca pugilator*, were obtained from the Gulf Specimen Co., Panacea, Florida, and acclimatized for three days to the laboratory conditions under 12:12 L:D in a recirculating artificial seawater system.

Tissue preparation and homogenization for 5-HT determinations

In this section and the following two, we describe the procedure for determining the 5-HT concentrations in the components of the central nervous system of crayfish maintained in the stock tanks. The eyestalks, brain, subesophageal ganglion, thoracic ganglia, and abdominal nerve cord of 100 crayfish were dissected out as rapidly as possible in cold Van Harreveld's crayfish physiological saline (van Harreveld, 1936). These components were then distributed, ten per tube, in tubes containing 1 ml of 0.4 *M* perchloric acid and sonicated in four cycles of 30 s each with a sonicator (Biosonik-II, Bronwill Scientific) equipped with narrow probe for small volumes. The homogenates were centrifuged (10,000 × *g*) for 15 min at 4°C. The pH of the supernatant, after decanting, was adjusted to 6.0 with 2 *M* potassium carbonate, and the mixture was again centrifuged for 10 min; the supernatants were used for further purification. The wet weight of each tissue was recorded. The averages for the ten determinations of the 5-HT contents of each nervous system component were then calculated.

Purification and analysis of the sample

The supernatants were filtered and purified on a weakly acid cation exchanger column, Amberlite IRP-64 (Hansson and Rosengren, 1978). The column was glass, 30 mm long with 5 mm i.d., equipped with a Millipore HV 0.45 μm filter (Nihon Millipore Kogyokk) at the bottom and filled with 300 μl of the resin. The supernatant from each 1 ml extract was divided into two 500 μl aliquots, and each aliquot was loaded separately onto the resin at a rate of 50 μl/min. The 5-HT adsorbed onto the resin from each aliquot was eluted with 500 μl of 1.2 *N* HCl. The two eluted fractions were combined for faster analysis of only one sample rather than two, and we obtained high recovery which was always in the range of 80–82%. The data presented have been corrected to reflect the recovery percentage. The remaining samples were stored at −70°C (Hansson and Rosengren, 1978; Elofsson, *et al.*, 1982).

A Waters RP-HPLC unit, Model 501, fitted with a U6K universal LC injector and a 3.9 × 150 mm 5 μm silica C-18 steel column with a small guard column, coupled to a Waters electrochemical detector (Model 460) was used for the quantitative analysis of 5-HT in the samples. Five aliquots (25 μl) of each sample were run, and the results were averaged. The variation among samples was less than 10%. The elution reagent was methane sulphonic acid (40 m*M*) and phosphoric acid (30 m*M*) in 17% methanol, pH 2.5, and was thoroughly degassed before use (Hansson and Rosengren, 1978; Elofsson *et al.*, 1982; Nässel and Laxmyr, 1983). The pressure applied was 1500 psi with a flow rate of 1 ml/min. The detector was a glassy carbon electrode, and the working potential was set at +0.75 V against the reference electrode. The 5-HT concentrations were determined by comparing the peak height in the elution profile of the sample with that of the standards, and are presented as pg/mg of wet tissue. One additional criterion, other than elution time, was used to identify the 5-HT peak in the samples. Before analysis, we added a small amount (10 μg/ml) of synthetic 5-HT to the samples. In no case did the sample peak show any inhomogeneity due to the addition of 5-HT to the biological material when compared with the peak of the standard. With this analytical system we could detect as little as 25 pg of 5-HT.

Calibration curve

The calibration curve was prepared as follows. To nine clean glass tubes, each containing 2 ml of 0.4 *M* perchloric acid, was added a known amount of 5-HT creatinine sulfate monohydrate (0–51.2 ng/ml free base) and 10^{-5} *M* 3,4-dihydroxybenzylamine hydrobromide (DHBA) (internal standard). The samples were loaded onto the ion exchange column (Amberlite IRP-64) and treated as above. Samples (100 μl) were collected and 25 μl of the eluate was injected onto the RP-HPLC column. The peak heights were recorded and fitted in a graph against the concentration of 5-HT free base. The retention time, with 1 ml/min flow rate and at 1500 psi, was 8.8–8.9 min for 5-HT and 3.8 min for DHBA (Fig. 1).

Experimental protocols

The 5-HT content in the central nervous organs of the crayfish were initially determined using specimens that had been exposed for 2 days to 12:12: L:D. In addition, 20 equal-sized crayfish were held continuously for an additional three days either under a fluorescent light (450–500 lux) or in darkness. After these three days, all of the central nervous organs from ten crayfish were dissected out for 5-HT determinations. In addition, the central nervous organs were removed from the rest of these crayfish and homogenized in 2 ml of crab physiological saline (Cooke *et al.*, 1977), pH 7.4. These extracts were then centrifuged (10,000 × *g*) at 4°C, and the supernates were bioassayed for red pigment-dispersing activity in eyestalkless fiddler crabs, *Uca pugilator*, of 10–15 mm carapace width. The pigment in the erythrophores of these eyestalkless crabs was initially maximally concentrated. The erythrophores were staged according to the method

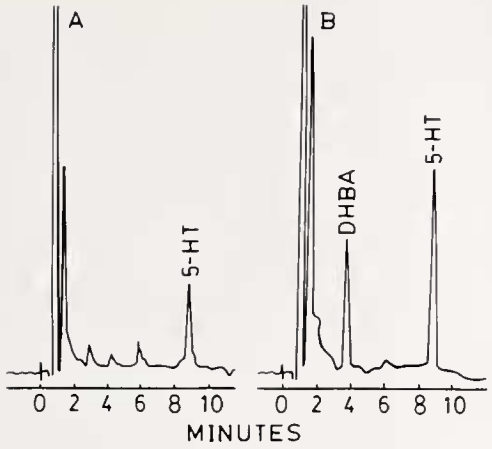

Figure 1. Chromatograms of (A) brain supernatant of *Procambarus clarkii* and (B) aqueous standard containing 5-HT and DHBA. Column: 5 μm silica C-18 steel. Mobile phase: methane sulphonic acid and phosphoric acid in methanol. Detector: glassy carbon electrode at +0.75 V potential. Retention time for 5-HT 8.8–8.9 min and for DHBA 3.8 min.

of Hogben and Slome (1931) wherein stage 1 indicates maximal pigment concentration, stage 5 maximal pigment dispersion, and stages 2, 3, and 4 the intermediate conditions. The Hogben and Slome stages for the experimental and control animals were then used to calculate Standard Integrated Responses (SIR) of the erythrophores of *Uca pugilator* to the nervous tissue extracts of *Procambarus clarkii* according to the method of Fingerman *et al.* (1967). Briefly, when pigment dispersion occurs, the sum of the Hogben and Slome stages recorded for the duration of the experiment for the control group is substracted from the corresponding sum for the experimental group. The difference is the SIR. The SIR integrates the amplitude, which is based on the observed Hogben and Slome stages, and duration of the response of the erythrophores. A dose of 50 μl containing the tissue extract equivalent to either one eyestalk, brain, subesophageal, or thoracic ganglion was injected into each crab.

For experiments involving *in vitro* electrical stimulation, the entire optic tract, including the major ganglia and sinus gland, was removed from the eyestalk and maintained in 50 μl physiological saline. The remaining nervous tissue from the brain to the end of thoracic nerve cord was also dissected out intact and carefully placed in 100 μl of physiological saline. Electrical stimulation of the isolated tissue was performed as described in detail by Quackenbush and Fingerman (1984). Briefly, the eyestalk tissue was held in place with a suction electrode attached to the stump of the optic nerve, whereas the central nerve tract, from the brain to the end of the thoracic nerve cord, was held in place by a suction electrode attached at the brain end. The stimulation given via the suction electrode was 5 pps, 4 ms delay, with pulses of 40 ms duration and varying voltage (10, 15, 20, and 25 V). Stimulation was delivered by a stimulator (Model S44) with a stimulus isolation unit (Model SIU 5A; both from the Grass Instrument Co.) After a stimulation bout of 2 min, the nervous tissues were homogenized in 0.4 *M* perchloric acid and processed for 5-HT determination as described above. A total of 75 crayfish were used, divided equally among one group of unstimulated controls and four groups of voltage stimulated preparations.

Experiments on the effects of pharmacological agents, such as fenfluramine (5-HT releaser, Consolo *et al.*, 1979), fluoxetine (5-HT potentiator, Wong *et al.*, 1975), 5,6-dihydroxytryptamine (5,6-DHT) (5-HT neurotoxin, Baumgarten *et al.*, 1982), and reserpine (5-HT depletor, Myhrberg *et al.*, 1979; Elofsson *et al.*, 1982), on the levels of 5-HT in the central nervous tissues of *Procambarus clarkii*, were performed according to the procedure of Myhrberg *et al.* (1979). Crayfish were divided into 5 groups of 15 each. The first group received physiological saline alone and served as the control. The crayfish in the second through fifth groups were administered various concentrations (10–25 μg/g body weight) of either fenfluramine, fluoxetine, 5,6-DHT, or reserpine. All injections were given once in a dose of 50 μl, and the crayfish were sacrificed after 2 h and their nervous tissues removed and processed for 5-HT analysis as described earlier.

Fenfluramine hydrochloride, 5,6-DHT, reserpine, and Amberlite IRP-64 were purchased from Sigma. Fluoxetine hydrochloride was a gift from Lilly Research Laboratories, whereas the 5-HT creatinine sulfate monohydrate and DHBA were purchased from Aldrich. All drugs were dissolved in isosmotic crayfish physiological saline.

The data obtained from these experiments were analyzed statistically by calculating the standard error for each of the means (SEM).

Results

The measurements quantifying 5-HT in the central nervous organs, eyestalks, brain, subesophageal ganglion, thoracic ganglia, and abdominal nerve cord of crayfish maintained under laboratory conditions (12:12 L:D; 24°C) in recirculating freshwater for two days are summarized in Figure 2. The 5-HT concentration was highest in the brain (168 pg/mg) and lowest in the abdominal nerve cord (54 pg/mg), with the eyestalk, subesophageal ganglion, and thoracic ganglia having intermediate concentrations. Because the concentration of 5-HT in the abdominal nerve cord is small relative to the rest of the central nervous organs, only eyestalks, brains, subesophageal ganglia, and thoracic ganglia were used in the rest of the experiments.

The pigment in the erythrophores of the crayfish (*P. clarkii*) that were illuminated while on a white background

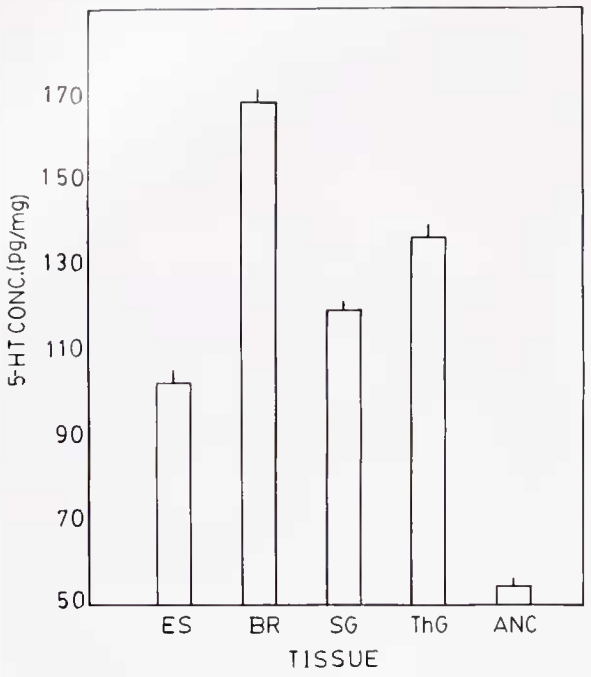

Figure 2. 5-HT concentration in pg/mg of tissue, assayed by HPLC and electrochemical detection, in eyestalks (ES), brain (BR), subesophageal ganglion (SG), thoracic ganglia (ThG), and abdominal nerve cord (ANC) of *Procambarus clarkii*. Error bars are SEM for ten separate extracts of tissue pooled from ten animals each.

was concentrated, whereas the red pigment of the crayfish kept in darkness was dispersed. The 5-HT concentrations in the eyestalks, brain, subesophageal ganglion, and thoracic ganglia of crayfish held under continuous light for three days on a white background increased significantly (Fig. 3). The corresponding red pigment-dispersing SIR values evoked in the fiddler crabs by these extracts also increased. In contrast, both the 5-HT levels and the SIR values in the eyestalks, brains, subesophageal ganglia, and thoracic ganglia of crayfish held in darkness showed significant decreases when compared to the controls (Fig. 3).

In the experiments in which eyestalk, neural ganglia, and the central nerve tract (from brain to the end of the thoracic nerve cord) were electrically stimulated with various voltages (10, 15, 20, and 25 V), 25 V was found to be most effective. The data in Figure 4 are for tissues stimulated with 25 V. The stimulation produced significant decreases in the concentration of 5-HT in all the tissues, with the maximum decrease occurring in the eyestalks (−37.4%) and the minimum in the thoracic ganglia (−22.8%). Furthermore, when extracts of the electrically stimulated tissues were bioassayed for red pigment-dispersing activity in crabs, it was found that the SIR values evoked by these extracts were significantly decreased in comparison to the tissue extracts from the control crayfish. Interestingly, the percentage decrease in 5-HT content decreased progressively in the tissues along the central nervous chain from the brain to the thoracic ganglia.

Of the concentrations tested, the smallest concentrations that produced significant effects on the 5-HT level after 2 h were 15 μg/g body weight of fenfluramine, 10 μg/g body weight of 5,6-DHT, and 15 μg/g body weight of reserpine. Fenfluramine induced a significant decrease of 5-HT from all of the nervous tissues (Fig. 5). The decrease was maximum in the eyestalks and least in the thoracic ganglia. None of the concentrations (10–25 μg/g body weight) of fluoxetine produced any significant effect on the 5-HT concentration in any of the tissues. 5,6-DHT and reserpine produced significant decreases in the 5-HT concentration of all the nervous tissues tested. The maximum decrease was produced in the eyestalks, whereas the minimum decrease occurred in the thoracic ganglia.

Discussion

Histochemical studies by means of fluorescence microscopy have revealed the presence of yellow-fluorescing

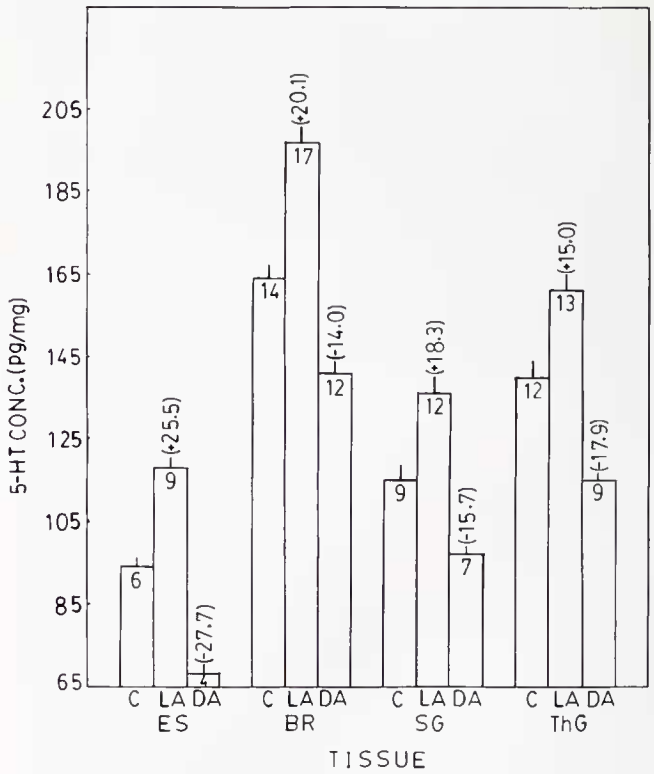

Figure 3. 5-HT concentration in eyestalks (ES), brain (BR), subesophageal ganglion (SG) and thoracic ganglia (ThG) of *Procambarus clarkii* exposed for three days to continuous light (light adapted, LA) or held in total darkness (dark adapted, DA). Error bars are SEM of ten separate extracts of tissues pooled from ten animals each. Figures in parentheses are percent change from the 12:12 L:D control (C) and those in the columns are the red pigment-dispersing Standard Integrated Responses (SIR) of the erythrophores of eyestalkless fiddler crabs, *Uca pugilator*, to extract of that tissue of *Procambarus clarkii*.

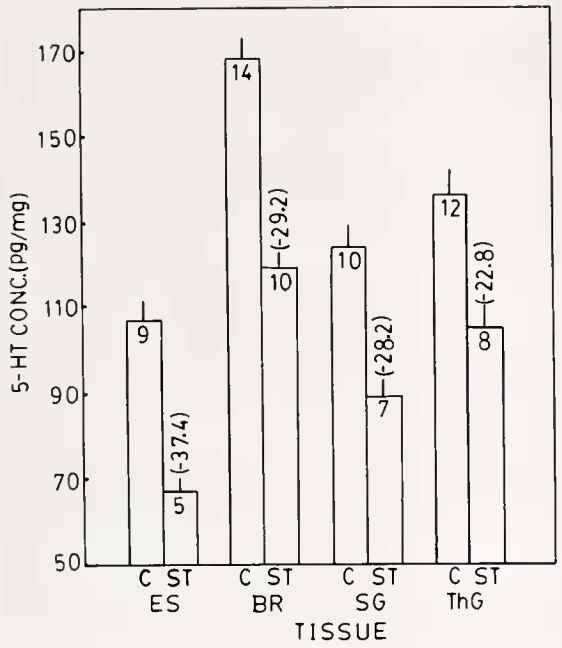

Figure 4. Effect of 25 V electrical stimulation (ST) for 2 min on the concentration of 5-HT in eyestalks (ES), brain (BR), subesophageal ganglion (SG), and thoracic ganglia (ThG) of *Procambarus clarkii*. Error bars are SEM of ten separate extracts of tissues pooled from ten animals each. Figures in parentheses denote percent change from the control (C) and those in columns are the red pigment-dispersing Standard Integrated Responses (SIR) of the erythrophores of eyestalkless fiddler crabs, *Uca pugilator*, to extracts of that particular tissue of *Procambarus clarkii*.

cells, indicative of 5-HT, in the eyestalks, brain and ventral nerve cord of the crayfishes, *Astacus astacus* (Elofsson *et al.*, 1966) and *Pacifastacus leniusculus* (Myhrberg *et al.*, 1979). The identification of biogenic amines and the determination of their concentrations in arthropods had earlier depended on fluorometric methods. Those methods required relatively large tissue samples and yielded readings only in the microgram or nanogram range. The advent of HPLC technology enabled investigators to analyze the 5-HT content of tissues from small arthropods with only moderate amounts of biogenic amines, and the sensitivity has been extended down to the picogram level.

A comparison of the amount of 5-HT present in various tissues of the central nervous system of the red swamp crayfish, *Procambarus clarkii*, revealed distinct differences among them (Fig. 2). These experiments provide, for the first time, data on the amount of 5-HT in different nervous tissues of this crayfish. The level of 5-HT found in the eyestalks (102 pg/mg) of *Procambarus clarkii* is comparable to the value reported by Elofsson *et al.* (1982) for the eyestalks (100 pg/mg) of another crayfish, *Pacifastacus leniusculus*, whereas the brain (168 pg/mg) of *Procambarus clarkii* contained slightly more 5-HT than the brain (150 pg/mg) of *Pacifastacus leniusculus* (Elofsson *et al.*, 1982), but like *Pacifastacus* the 5-HT level in the brain of *Procambarus* is higher than in the eyestalk.

The 5-HT levels in all nervous tissues of crayfish held for three days under constant illumination on a white background were higher than the corresponding values for the crayfish held for three days in complete darkness, and the values of the control crayfish held under 12:12 L:D (Fig. 3). Furthermore, the red pigment-dispersing SIR values for the erythrophores of the eyestalkless fiddler crabs, *Uca pugilator*, that received the extracts of eyestalks, brain, subesophageal ganglion, and thoracic ganglia of the light adapted crayfish were also significantly higher than the corresponding SIR values evoked by the nervous tissues of the dark adapted crayfish or by those of the controls. Earlier studies with the fiddler crab, *Uca pugilator*, and dwarf crayfish, *Cambarellus shufeldti*, had revealed that 5-HT functions as a neurotransmitter that stimulates the release of red pigment-dispersing hormone (Rao and Fingerman, 1970, 1975). The changes that occurred in the 5-HT concentrations in the central nervous organs of the crayfish in darkness or in constant illumination on a white background presumably reflect this role of 5-HT in releasing the color change hormone. The red pigment of the crayfish kept in light on a white background was concentrated. The central nervous tissues of these crayfish on

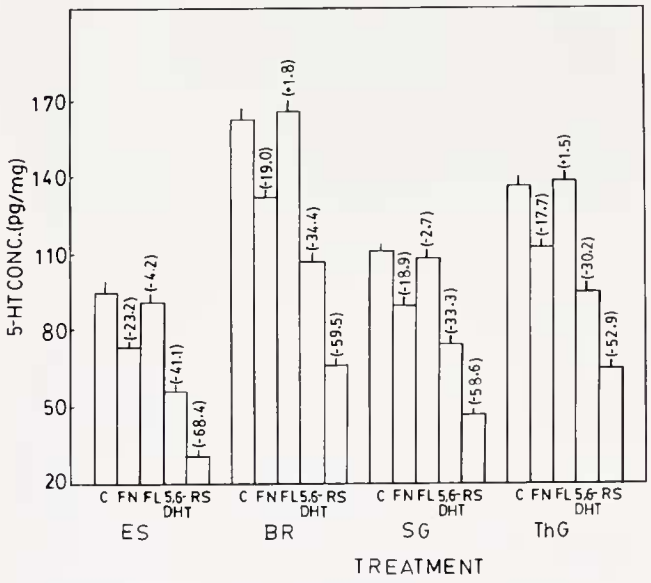

Figure 5. Effects of pharmacological agents on the concentration of 5-HT in eyestalks (ES), brain (BR), subesophageal ganglion (SG), and thoracic ganglia (ThG) of *Procambarus clarkii*. Error bars are SEM of ten separate extracts of tissues pooled from ten animals each. Values in parentheses show the percent change from the control (C). 5,6-DHT = 5,6-dihydroxytryptamine, 10 μg/g body weight; FN = fenfluramine, 15 μg/g body weight; FL = fluoxetine, 25 μg/g body weight; RS = reserpine, 15 μg/g body weight. The volume of drug solution or saline injected was 50 μl. The tissues were removed from the crayfish 2 h after the injections were given.

a white background not only contained higher 5-HT levels than the tissues of the control crayfish, but also evoked higher red pigment-dispersing SIR values, observations that are consistent with a pigment-dispersing hormone releasing role of 5-HT. Presumably, because the red pigment was concentrated, neither 5-HT nor pigment-dispersing hormone was being used, thereby accounting for the increased levels of both substances. Earlier, Fingerman *et al.* (1964) reported the presence of erythrophorotropic hormones in the eyestalks and brain of juveniles and adults of *Procambarus clarkii*. They observed that the injection of an extract containing one-third of an organ complement per dose significantly evoked pigment migration in eyestalkless animals. More recently, McCallum *et al.* (1988, 1989) confirmed the previous findings of Fingerman *et al.* (1964) by isolating and sequencing the pigment-dispersing hormone (PDH), which is an octadecapeptide, from the eyestalks of *Procambarus clarkii*.

Rao and Fingerman (1975) later reported that 5-HT, when injected into the dwarf crayfish *Cambarellus shufeldti*, dispersed the red pigment in the erythrophores, as in the fiddler crab, but was ineffective when tested *in vitro* on isolated chromatophore-bearing pieces of the crayfish carapace. In crayfish on a white background with their red pigment concentrated, 5-HT turnover would presumably have decreased because, with the red pigment concentrated, 5-HT would not be used to stimulate release of red pigment-dispersing hormone, so a rise in the intraneuronal concentration of this neurotransmitter would occur. On the other hand, because darkness fosters red pigment dispersion, crayfish in darkness would be using the intraneuronal stores of 5-HT to effect red pigment dispersion and would, according to the hypothesis, have a lower intraneuronal concentration of 5-HT than crayfish on a white background under light, which the present data show is indeed the case.

Previously, Berlind and Cooke (1970) reported the release of a neurosecretory peptide hormone from the pericardial organs of the spider crabs *Libinia emarginata* and *Libinia dubia* following electrical stimulation. Later, Quackenbush and Fingerman (1984) found that electrical stimulation of the isolated eyestalks of the fiddler crab, *Uca pugilator*, releases chromatophorotropic peptides from the sinus gland. Recently, Kulkarni and Fingerman (1991) also used *Uca pugilator* to show that the distal retinal pigment light-adapting hormone is released by electrical stimulation of isolated eyestalk neuroendocrine tissues. The data presented in Figure 4 clearly show that 25 V stimulation reduced the 5-HT levels in the central nervous tissues of *Procambarus clarkii*. Furthermore, the stimulation appears also to have reduced the amount of stored red pigment-dispersing hormone in the central nervous system because the extracts of the stimulated tissues produced lesser SIR values for red pigment dispersion than did the tissues of the unstimulated controls. In their studies, both Quackenbush and Fingerman (1984) and Kulkarni and Fingerman (1991) bioassayed only the bathing fluid and not the actual stimulated tissue. In the present study, the stimulated tissues were extracted and bioassayed for red pigment-dispersing activity by injecting the extracts into eyestalkless crabs, *Uca pugilator*.

Fenfluramine (5-HT releaser), 5,6-DHT (5-HT neurotoxin), and reserpine (5-HT depletor) decreased the amount of 5-HT in the central nervous system, although their modes of action are different (Fig. 5). However, the 5-HT potentiator fluoxetine had no appreciable effect on the 5-HT concentration. These findings are consistent with earlier 5-HT depletion studies in which reserpine was used with crustaceans. Myhrberg *et al.* (1979), using the histochemical fluorescence method of Falck and Hillarp, and Elofsson *et al.* (1982), using HPLC, both found that reserpine decreases the 5-HT content of nervous tissues in the crayfish, *Pacifastacus leniusculus*. Likewise, the 5-HT content of the brain and eyestalks of *Uca pugilator* decreased after injection of reserpine and 5,6-DHT (Fingerman *et al.*, 1974).

Acknowledgments

This research was supported by Grant No. I-1435-88 from BARD, The United States-Israel Binational Agricultural Research & Development Fund. We thank Mr. Chayan Chakraborti for his technical assistance.

Literature Cited

Aréchiga, H., E. Banuelos, E. Frixione, A. Picones, and L. Rodriguez-Sosa. 1990. Modulation of crayfish retinal sensitivity by 5-hydroxytryptamine. *J. Exp. Biol.* **150:** 123–143.

Baumgarten, J. G., S. Jenner, A. Bjorklund, M. P. Klemm, and H. G. Schlossberger. 1982. Serotonin neurotoxins. Pp. 249–277 in *Biology of Serotonergic Transmission*, N. N. Osborne, ed. John Wiley & Sons, New York.

Beltz, B. S., and E. A. Kravitz. 1983. Mapping of serotonin-like immunoreactivity in the lobster nervous system. *J. Neurosci.* **3:** 585–602.

Berlind, A., and I. M. Cooke. 1970. Release of neurosecretory hormone as peptide by electrical stimulation of crab pericardial organs. *J. Exp. Biol.* **53:** 679–686.

Consolo, S., M. Ladinsky, A. S. Tirelli, V. Crunelli, S. Samanian, and S. Garattini. 1979. Increase in rat striatal acetylcholine content by d-fenfluramine, a serotonin releaser. *Life Sci.* **25:** 1975–1981.

Cooke, I. M., B. A. Haylett, and T. M. Weatherby. 1977. Electrically elicited neurosecretory and electrical responses of the isolated crab sinus gland in normal and reduced calcium salines. *J. Exp. Biol* **70:** 125–149.

Elofsson, R. 1983. 5-HT-like immunoreactivity in the central nervous system of the crayfish, *Pacifastacus leniusculus*. *Cell Tiss. Res.* **232:** 221–236.

Elofsson, R., T. Kauri, S. O. Nielsen, and J. O. Stromberg. 1966. Localization of monoaminergic neurons in the central nervous system of *Astacus astacus* Linné (Crustacea). *Z. Zellforsch. Mikroskop. Anat* **107:** 343–360.

Elofsson, R., L. Laxmyr, E. Rosengren, and C. Hansson. 1982. Identification and quantitative measurements of biogenic amines and DOPA in the central nervous system and haemolymph of the crayfish, *Pacifastacus leniusculus* (Crustacea). *Comp. Biochem. Physiol.* **71C:** 191–205.

Fingerman, M. 1985. The physiology and pharmacology of crustacean chromatophores. *Am. Zool.* **25:** 233–252.

Fingerman, M., W. E. Julian, M. A. Spirtes, and R. M. Kostrzewa. 1974. The presence of 5-hydroxytryptamine in the eyestalks and brain of the fiddler crab *Uca pugilator*, its quantitative modification by pharmacological agents, and possible role as a neurotransmitter in controlling the release of red pigment-dispersing hormone. *Comp. Gen. Pharmac.* **5:** 299–303.

Fingerman, M., K. R. Rao, and C. K. Bartell. 1967. A proposed uniform method of reporting response values for crustacean chromatophorotropins: the standard integrated response. *Experientia* **23:** 962–964.

Fingerman, M., Y. Yamamoto, and C. W. Jacob. 1964. Differences between the chromatophore systems of juvenile and adult specimens of the crayfish *Procambarus clarkii*. *Am. Zool.* **4:** 164.

Gerschenfeld, H. M. 1973. Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.* **53:** 1–119.

Hansson, C., and E. Rosengren. 1978. Quantitative analysis of 5-hydroxytryptamine in biological material by high performance liquid chromatography and electrochemical detection. *Anal. Lett.* **B11:** 901–912.

Hogben, L., and D. Slome. 1931. The pigmentary effector system-VI. The dual character of endocrine co-ordination in amphibian colour change. *Proc. R. Soc. Lond.* **108B:** 10–53.

Krnjevic, K. 1974. Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* **54:** 418–540.

Kulkarni, G. K., and M. Fingerman. 1991. Release of black pigment-dispersing hormone and distal retinal pigment light-adapting hormone upon electrical stimulation of the isolated eyestalk neuroendocrine complex of the fiddler crab, *Uca pugilator*. *Comp. Biochem. Physiol.* **99C:** 47–52.

Kulkarni, G. K., R. Nagabhushanam, G. Amaldoss, R. G. Jaiswal, and M. Fingerman. 1991. 5-hydroxytryptamine stimulation of the ovary in the crayfish, *Procambarus clarkii*. *Am. Zool.* **31:** 115A.

Laxmyr, L. 1984. Biogenic amines and DOPA in the central nervous system of decapod crustaceans. *Comp. Biochem. Physiol.* **77C:** 139–143.

McCallum, M. L., K. R. Rao, and J. P. Riehm. 1989. A comparison of the effects of two pigment-dispersing hormones on the crayfish *Procambarus clarkii*. *Am. Zool.* **29:** 217.

McCallum, M. L., K. R. Rao, J. P. Riehm, C. J. Mohrherr, and W. T. Morgan. 1988. Isolation of a β-PDH analog from the crayfish, *Procambarus clarkii*. *Biol. Bull.* **177:** 225–229.

Myhrberg, H. E., R. Elofsson, R. Aramant, N. Klemm, and L. Laxmyr. 1979. Selective uptake of exogenous catecholamines into nerve fibres in crustaceans. A fluorescence histochemical investigation. *Comp. Biochem. Physiol.* **62C:** 141–150.

Nässel, D. R., and L. Laxmyr. 1983. Quantitative determination of biogenic amines and DOPA in the CNS of adult and larval blowflies, *Calliphora erythrocephala*. *Comp. Biochem. Physiol.* **75C:** 259–265.

Quackenbush, L. S., and M. Fingerman. 1984. Regulation of the release of chromatophorotropic neurohormones from the isolated eyestalk of the fiddler crab, *Uca pugilator*. *Biol. Bull.* **166:** 237–250.

Rao, K. R., and M. Fingerman. 1970. Action of biogenic amines on crustacean chromatophores, II. Analysis of the responses of erythrophores in the fiddler crab, *Uca pugilator*, to indolealkylamines and an eyestalk hormone. *Comp. Gen. Pharmacol.* **1:** 117–126.

Rao, K. R., and M. Fingerman. 1975. Action of biogenic amines on crustacean chromatophores, IV. Analysis of the synergistic erythrophoric pigment dispersion evoked by 5-hydroxytryptamine and lysergic acid diethylamide in the dwarf crayfish, *Cambarellus shufeldti*. *Comp. Biochem. Physiol.* **51C:** 53–58.

Reddy, T. S. N., G. K. Kulkarni, L. Glade, and M. Fingerman. 1990. Molt stages of the red swamp crayfish, *Procambarus clarkii*. *Am. Zool.* **30:** 109A.

Sandeman, R. E., and D. C. Sandeman. 1987. Serotonin-like immunoreactivity of giant olfactory interneurons in the crayfish brain. *Brain Res.* **403:** 371–374.

Sandeman, D. C., R. E. Sandeman, and A. R. Aitken. 1988. Atlas of serotonin-containing neurons in the optic lobes and brain of the crayfish, *Cherax destructor*. *J. Comp. Neurol.* **269:** 465–478.

van Harreveld, A. 1936. A physiological solution for fresh water crustaceans. *Proc. Soc. Exp. Biol. Med.* **34:** 428–432.

Werman, R. 1966. Criteria for identification of a central nervous system transmitter. *Comp. Biochem. Physiol.* **18:** 745–766.

Wong, D. T., F. P. Bymaster, J. S. Horng, and B. B. Molloy. 1975. A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *J. Pharmacol. Exp. Ther.* **193:** 804–811.